

The University of Chicago

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EXCITATION OF IMPAIRED SINGLE
MUSCLE FIBERS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
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MEMBRANE POTENTIALS AND EXCITATION OF IMPALED SINGLE MUSCLE FIBERS¹

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SIX FIGURES

These studies, begun in 1940 and reported briefly in 1942 (Graham, Carlson and Gerard, '42; Gerard and Graham, '42) were interrupted by the war and are still incomplete. Since they were initiated independently of the microelectrode studies on the giant axone by Hodgkin and Huxley ('39, '45) and Cole and Curtis ('40), and supplement that evidence on a different tissue, it seems desirable to put the present findings on record.

The giant fiber, up to a millimeter in diameter (Curtis and Cole, '40) is admirable for internal exploration with a microelectrode, since this can be inserted longitudinally and the tip pushed far from the region of penetration and damage. These fibers are available, however, only at restricted seasons and localities and after painstaking preparation. Striated muscle fibers, about 70 micra in diameter (Curtis and Cole, '42), are always available with little trouble and in great number and, as was hoped, proved to be satisfactory for many purposes. Contractility offers another variable for correlation with the usual membrane properties and is a convenient index of response. It is also, unfortunately, a source of trouble, for movement of the fiber increases the damage produced by a radially inserted microelectrode. An oblique insertion minimizes this. This report deals primarily with

¹The present investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

potentials measured and electric stimuli delivered across the membranes of single fibers of the frog sartorius muscle.

METHOD

Tissue. A frog sartorius was dissected without touching the muscle mass and with no twitch except as the nerve is cut. The deep surface separates readily and with minimal covering fascia. It offers some hundred intact muscle fibers for impaling, with no further dissection. The muscle was mounted, deep surface up, on a paraffin block with a central glass window. Its tendinous ends were fixed by pins in the paraffin, the muscle being under slight stretch, and the block placed on the stage of a dissecting microscope for observation at 50 times magnification. Sufficient illumination by transmitted light is obtained from an ordinary desk lamp. To prevent heating and drying of the preparation, a shallow paraffin wall was used around the muscle and a pool of Ringer or other solution formed. This was renewed frequently by drip from a reservoir and drainage through a capillary. A muscle remains in good condition most of a day.

Electrodes. Glass capillary tubing about 1 mm in diameter was drawn to a microtip in the usual manner, bent at a right angle, and cemented into the end of a glass tube of dimensions convenient to a micromanipulator. The open end of this tube, bent to parallel the capillary tip, dipped into a beaker bridged to a calomel half-cell. The beakers and cells for both electrodes were placed in a metal shield with a switch for connecting them with the amplifier input or with the stimulator. In some recent experiments, arranged for injection, the tip was cemented into a hypodermic syringe with a Ag-AgCl lead sealed through the wall of the barrel. This seems to be as satisfactory as the calomel. Each electrode was filled with isotonic KCl and, when not in use, was kept, with the tip immersed in a test tube of the same solution. The tip of a usable electrode was 5 micra or less outer diameter, 1 micron smaller internal diameter, and occasionally a sufficiently stiff tip of ca. 2 micra was obtained. (Contrary to

the usual assumption, these tips, drawn according to the standard procedure, were frequently open at the end and did not need to be broken off. Such tips made the best electrodes.) When a tip had broken in use, the electrode could serve as the indifferent member of the pair, but, in order to eliminate any siphoning from the Ringer bath through the electrode into the lower KCl beaker, an agar-KCl-filled indifferent electrode was found preferable. Resistance of a single electrode fell in the range of 7 to 10 megohms.

Manipulation. The indifferent electrode was placed by micromanipulation on or near the muscle, either within a millimeter of or at some distance from the active one. This latter was separately maneuvered into position over the desired fiber and the tip inserted by lowering it under visual control. The larger tips produced dimpling of the membrane before penetrating, but only rarely did insertion evoke a contraction. The small tips penetrated with no visible sign and the most certain evidence of entry was the abrupt potential change. After the initial adjustments, the electrode could be inserted or reinserted in fiber after fiber in a few seconds.

These capillary electrodes served to lead potentials from a fiber, to lead stimulating currents in and, in a few instances, to inject liquids. They could be transferred promptly from one function to another. A series of spike or saw-tooth shocks was obtained from a thyatron stimulator; single stimuli, from condenser discharges. Time-intensity curves were determined in the usual way, with a 10,000 ohm fixed resistance shorting the electrodes.

Amplifier. A direct current gain, modified Wynn-Williams amplifier was used for most of this work. A 900 megohm input resistor made electrode resistance negligible; an RCA 954 pentode with balanced bias and output as used with a standard galvanometer gave a deflection of about 3 mm per mV. Stability was adequate. This has now been replaced by the new Victoreen Vw32 electrometer tube which, using an ordinary wall galvanometer (sensitivity 8×10^{-9} amps. per mm at 1 M; resistance 1150 ohms), at 1/10 sensitivity, gives

2 mm per mV. Stability is excellent and both circuit and power supply are much simpler (fig. 1). Mr. George R. Carlson has been most helpful in suggesting and building the amplifiers.

Checks. With both electrodes in ringer solution, and a correction made for the imbalance in the calomel half cells, the usual P.D. observed was only a fraction of a mV. Difference in size of electrode tips had no effect. Larger deflections, occasionally encountered, were eliminated by grounding the amplifier, calomel cell shield, microscope, etc.

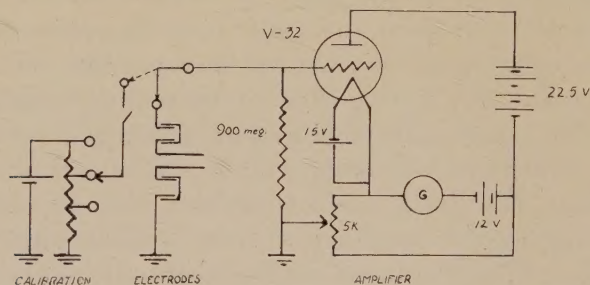


Fig. 1 Amplifier circuit.

An unmeasured difference in boundary potential is, of course, introduced between the electrode and ringer on the one hand and the electrode and myoplasm on the other, when a fiber is impaled. In the comparable case of the giant axone, this has been estimated at 10 mV (Curtis and Cole, '42); and a similar value may be assumed for the muscle fiber. This is small relative to the measured membrane potentials and in such a direction as to make these measurements less than the true values.

RESULTS

A. The membrane potential

1. *Absolute magnitude.* The membrane potential was observed in several hundred fibers and recorded for sixty-five typical fibers in twenty muscles. The average value was 61.8 mV with a range from 41.0 to 80.4 mV. In any one muscle,

the greatest range from fiber to fiber was 20%. The higher values, on the whole, were obtained in later experiments with optimal technique. With a 10 mV correction for boundary potentials, the greatest membrane potential found would thus be 90 mV.

2. *Sources of error.* The variability in potential is presumably traceable to two factors: the original condition of the fiber, and the amount of injury produced by insertion of the electrode. Muscles from frogs in bad physical condition (slug-gish, red-leg), and especially during the summer, showed a characteristic slight opacity and their fiber potentials were in the lower range. A similar opaque appearance slowly develops if, as in some earlier experiments, the muscle is inadequately protected from heat or evaporation, and this also is accompanied by a lowered potential. Again, if the diameter of an electrode is too large for smooth penetration, causing considerable dimpling or even sidewise motion of the fiber during insertion, potentials are not likely to be in the top bracket; while, conversely, a particularly fine electrode which allows very smooth penetration gives high readings. Tearing of the membrane immediately surrounding the inserted electrode is able, thus, to lower the potential measured, at once and permanently.

3. *Time course.* In seven experiments with the electrode left in place, the recorded membrane potential fell along a smooth curve to three-quarters of its original value in 12, 15, 15, 20, 45, and 50 minutes respectively. The rate of fall is apparently very sensitive to the conditions of penetration; and in current experiments by Mr. Gilbert Ling, using an oblique insertion and fine needle, the initial potential has been maintained indefinitely in about half the cases. The accompanying graph presents a typical curve of fall and, for comparison, that of Du Bois Reymond (Heilbrunn, '43) for the time fall of the usual injury potential at an injured surface (fig. 2). The decrease of injury potential is due to a sealing over of the cut, so gradually excluding the active lead from the inside of the fibers (Gerard, '30), and it can be fully

restored by a fresh cut. In the present experiments, however, withdrawal and reinsertion of the active electrode led to no change in the membrane potential, with a single exception which showed a slight rise. The potential fall in impaled fibers is thus a different phenomenon from that at a cut surface and does not involve "healing" around the tip. (The isotonic KCl in the electrode may help to prevent this.) The potential fall is in the membrane itself and probably documents a progressively spreading and intensifying injury to it, produced by a direct physical action of the electrode tip

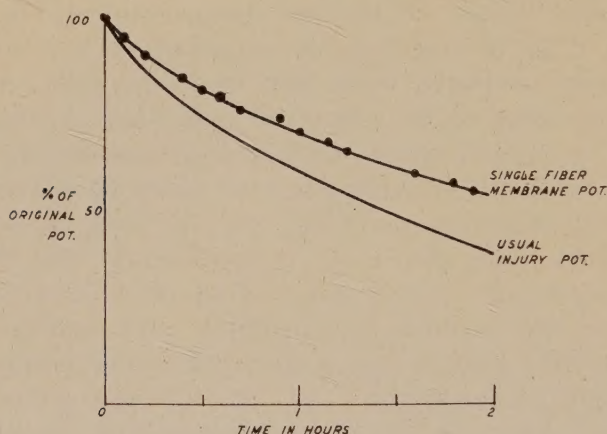


Fig. 2 Fall of potential after insertion of electrode.

or by depolarization resulting from ionic leakage around the inserted needle.

4. *Relation to injury potential.* The relation of the usually measured demarcation or injury potentials to the true membrane potential is clearly shown in a simple experiment. The pelvic half of the muscle is exposed to ether-Ringer, to produce a maintained injury, a vaseline barrier protecting the remaining half. An indifferent electrode (1) is placed on the normal surface, another (2) on the injured surface, and a micro-electrode (3) is inserted into a fiber in the normal region.

The following results of one experiment are typical also of three others with ether and of several with isotonic KCl.

ELECTRODES	POTENTIAL, mv
1-2	22.2
2-3	46.9
	69.1 Sum
1-3	67.2

Clearly, electrode two is tapping off only a fraction of the full membrane potential of an intact region.

The influence of a cut on the membrane potential was explored in more detail by measuring the potential recorded from the surface and the interior of single fibers, against an indifferent lead from the cut, at various distances from a

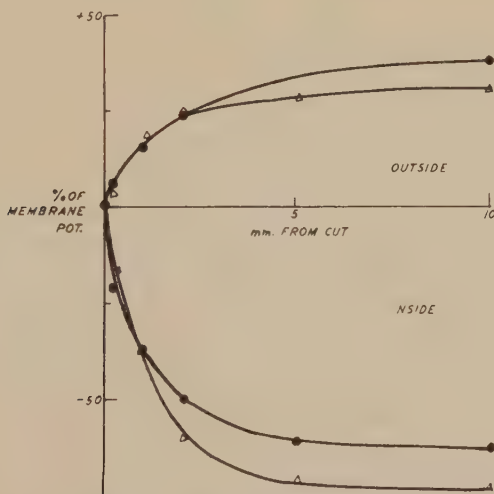


Fig. 3 Effect of injury on membrane potential

cut end. The average readings of several fibers are plotted for two separate muscles in figure 3. The curves show that at approximately 5 mm from a cut the membrane potential is at its normal value and that three-fourths of its fall to zero, at the cut, occurs within 2 mm of the cut. Further, the potential between a cut end and a normal surface is again seen to represent only a third (30% and 39% in these curves) of the full membrane potential.

It is obvious from such findings that the injured end of a muscle fiber makes no active contribution to the demarcation potential. The view that the "battery" is produced by the injury has been most recently urged by Sugi ('33) on the basis of an exhaustive exploration of the surface potential of muscles injured in various regions. We have made some effort to verify his findings but could not satisfy ourselves that our indifferent lead, distant in Ringer solution, was truly indifferent.

The significant question remains as to whether the decrease in membrane potential, as the cut is approached, is due to spreading injury to the membrane, so that it is generating less potential there, or whether the decrease represents rather the existence of an effective short circuit. In the latter case, the IR drop from outside to cut and from cut to inside will reflect the ratio of external to internal longitudinal resistance along the fiber. At 5 mm from the cut, presumably, the resistance from outside to cut to inside is no longer low compared to the local membrane leak and no significant longitudinal currents flow. At shorter distances, these looped currents increase. But the potential at the cut represents in all cases only one-third of the IR drop because the inside resistance is twice the outside one. The small diametered fiber core with a specific resistance greater than that of Ringer's fluid should have a greater resistance per unit length than should the surrounding intercellular fluid or bathing solution.

There remains, also, the probability that the membrane "battery" itself falls to zero as the cut is approached. This could be due directly to some spreading injury or indirectly to the current leak and maintained depolarization. The same problem exists in relation to the potential fall at the region of an inserted electrode. It seems unlikely that actual membrane damage spreads far from a region of mechanical injury: in fact, as mentioned, with a fine puncture the membrane potential measured right there can remain at full magnitude; and even after the usual puncture and withdrawing the

needle, the membrane potential remains normal 100 micra distant.

Addition of K^+ to the outside of a fiber lowers the membrane potential and injection of K^+ on the inside might be expected to raise it. In a few experiments, isotonic KCl or K_2HPO_4 or KCl with 10^{-3} acetylcholine was injected into fibers. With small volumes and minimal injury, little potential change resulted; with greater damage (fiber swollen and granular) the potential fell considerably. The findings are preliminary and do not exclude a potential increase under adequate conditions.

B. Transmembrane stimulation and response

1. *Current direction.* Since a muscle twitch is likely to tear the membrane around an inserted electrode, stimulation experiments were at first performed on isolated cut-end fibers (bullfrog adductor magnus), but it was later found possible to confirm these on the usual sartorius preparation. Contractions in the cut fibers were localized, those in the intact ones, propagated, but the response was obtained under like conditions in both cases. Typical results are shown in table 1.

TABLE 1

ELECTRODE POSITION		CURRENT THRESHOLD	
Anode	Cathode	Type	Voltage
Outside	Outside	Spike or condenser discharge	3
		Sawtooth	2
Inside	Inside	Spike or condenser	3
		Sawtooth	2
Inside	Outside	Spike or condenser	1
		Sawtooth	1
Outside	Inside	Make of constant current	2
		Break of constant current	∞ ¹
		Spike or condenser	∞
		Sawtooth	1
		Make of constant current	∞
		Break of constant current	3

¹ In arbitrary units for threshold response, " ∞ " means no response at over 100 times the unit threshold current. No higher voltage was available.

It is obvious that, with two symmetrical microelectrodes, whether they are both inside the fiber or both outside makes little difference. In each case, current flows both out and in across the membrane and the usual stimulus relations obtain.

With asymmetric leads, one inside and one outside the fiber, current crosses the membrane in only one direction. It is clear with the simple shock that a response can be obtained only when the current flow depolarizes the membrane (anode inside); a hundred-fold greater voltage in the reverse direction is ineffective. This ratio is great enough to more than offset any rectifying action at the membrane, as judged by the behavior of squid nerve (Cole and Hodgkin, '39; Cole, '41). With constant currents, excitation results only when the change is such as to decrease membrane polarization — on make with the anode inside, on break with the cathode inside. The response in both directions with the saw-tooth stimuli, having a relatively slow current build up, probably represents a combination of such make and break stimulations.

2. *Current intensity.* The absolute current flow across the membrane, required for excitation, should be obtainable for this preparation, as well as relative values for different conditions. The relative thresholds indicated above are in the order to be expected, except perhaps for the symmetrical and low values with the saw-tooth stimuli. The absolute current has not been determined for standardized conditions. It varies with shape and duration (capacity of condenser), with electrode placement (see below), and with other unexplored factors. The order of magnitude is indicated by these figures: 10^{-10} coulombs with a 0.01 mF condenser discharge, 3×10^{-9} with a 2.0 mF condenser.

3. *Time-intensity curves.* Time-intensity curves were obtained for six different fibers in two muscles, using transmembrane stimulation. A typical one is shown in figure 4. The shape is the familiar one obtained with external electrodes and the chronaxie value (aver. 0.4 msec.; range 0.30 to 0.48) is well in the normal range (0.2 to 0.5 msec.; Davis, '23) for

the sartorius muscle. The absence of any striking difference suggests that the factors affecting chronaxie (excitability, decay of excitability, current density) are involved in the same manner in transmembrane and in surface stimulation by microelectrodes.

4. *Membrane area.* With one electrode just inside a fiber and the other on the membrane surface above, the maximum density of current traverses the membrane. Yet responses can be obtained at lower voltages when the outer electrode is

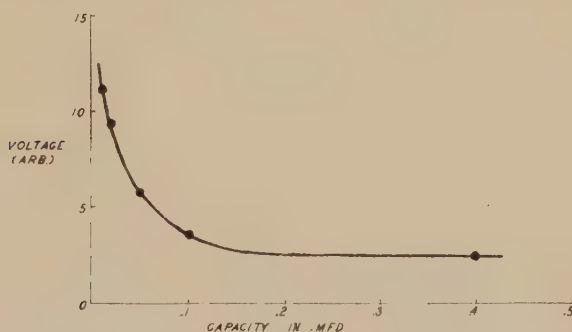


Fig. 4 Voltage-capacity curve for transmembrane stimulation.

moved some distance away from the membrane (table 2). The more distant electrode permits current to fan out through a greater area of membrane, and obviously the increased area of depolarization is more important to effective excitation than is the decreased current density. The character of the contraction is further evidence for the need of a minimal area of depolarization to set up a propagated membrane response. Contractions obtained with the outer electrode on the fiber surface are often local while those with the outer electrode at a distance are always fully propagated responses. The observations are repeatable in any order on a fiber and the differences with electrode position cannot be due to injury. The results confirm the earlier observation of Gelfan and Gerard ('30) and are in accord with their interpretation and with Rushton's analysis ('37).

Attempts have been made to compare excitation threshold and membrane potential values for single fibers, as these were varied by applied drugs and in other ways. Such data should help decide whether a response is initiated by a critical amount or level of depolarization or by other factors (impedance change, etc.). Results so far are not inconsistent with such a relationship but are too fragmentary to mean much.

TABLE 2
*Threshold*¹

OUTER ELECTRODE ON FIBER SURFACE	OUTER ELECTRODE 2 MM ABOVE SURFACE
16	14
12	7
15	13
10	8
12	12
9	6
8	8

¹ In arbitrary units. Each pair of readings on one fiber.

5. *The action potential.* The high resistance of these micro-electrodes would seriously distort rapid action potentials and such direct measurements have not been attempted. Indirect evidence has been obtained, however, that during action the membrane potential reverses, as in nerve. The whole muscle was kept tetanized through its nerve, stimulated at a given frequency, and the membrane potential obtained on a number of responding fibers by rapid insertion of the active electrode. The average of these potentials, at each frequency of tetanization, was compared with that for a group of resting fibers in the same muscle. Results are shown in table 3.

TABLE 3

FREQUENCY/SEC.	AVERAGE MEMBRANE POTENTIAL (mV)
0	63.0
50	53.5
100	38.5
150	34.6
190	26.8

This method of recording gives, of course, a time average of the changing and resting membrane potential rather than an individual action potential; and it is clear that, as the rate of stimulation is increased, the time during which the membrane is at its resting potential will decrease so that the measured value will approach an average of the action potential. Thus, assuming an approximate duration of 2 msec. for the monophasic action spike (Cole, '41), at a frequency of 100 stimuli/sec., the membrane is presumably at its resting potential for about 80% of the time and undergoing action potentials for 20%; while at 200/sec. the figures are 60% and 40%. With an assumed average value for the action potential and the known resting potential, it is thus possible to compute the measured potential at each frequency; or, conversely, from the measured potential the average value of the action potential can be calculated. Obviously many assumptions are involved and the results are only suggestive. Yet it is clear (table 4) that a good fit is obtained for an average action potential of -30 mV; while $+30$ mV gives bad discrepancies. Yet if the action spike reached only to zero potential, the average would be about $+30$. To average -30 would require the action potential to become negative (reverse) by 123 mV, or about 200%; a value similar to that found for nerve (Hodgkin and Huxley, '45; Curtis and Cole, '42).

TABLE 4

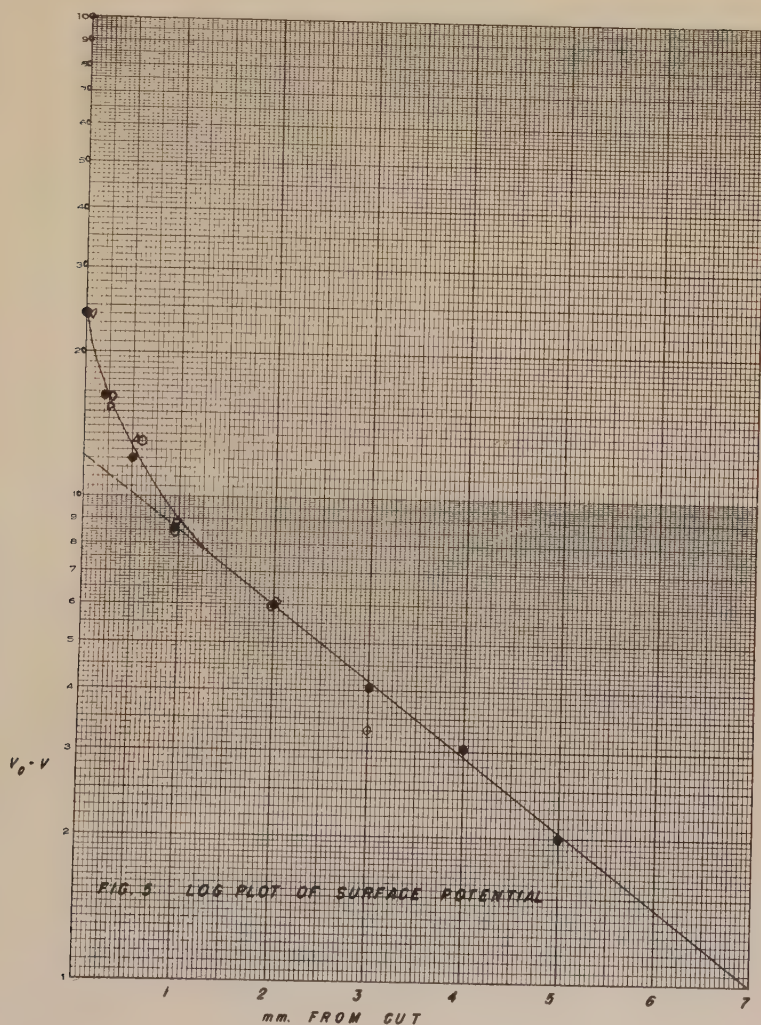
FREQ./ SEC.	FRACTION OF TIME AT RESTING POTENTIAL	FRACTION OF TIME AT ACTION POTENTIAL	POTENTIAL CONTRIBUTION OF RESTING MEMBRANE (Column 2 $\times$ 63 mV)	POTENTIAL CONTRIBUTION OF ACTION POTENTIAL (Column 3 $\times$ aver. assumed)		SUM		OB- SERVED
				+ 30 mV (5a)	- 30 mV (5b)	(4) + (5a)	(4) + (5b)	
(1)	(2)	(3)	(4)					
0	1.0	0.0	63	0	- 0	63	63	63
50	.9	.1	57	3	- 3	60	54	54
100	.8	.2	50	6	- 6	56	44	39
150	.7	.3	44	9	- 9	53	35	35
200	.6	.4	38	12	- 12	50	26	27

DISCUSSION

The average resting membrane potential measured for frog sartorius muscle fibers is 62 mV, the highest, 80 mV. Both values must be corrected for an opposed liquid junction potential, presumably about 10 mV, to 72 mV average and 90 mV maximum. This average is greater than that reported (Hodgkin and Huxley, '39; Curtis and Cole, '42) for squid axone, 51 mV, and still includes low values due unquestionably to injured or unhealthy fibers. That the frog muscle potential is normally higher than that of the squid nerve cannot be asserted from the limited data at hand; but it is consistent with such a view that the ratio of K^+ inside to K^+ outside is greater for the muscle, 35 to 1 (Fenn, '36), than the squid axone, 18 to 1 (Bear and Schmitt, '39). For these ratios, the diffusion potential should be 90 mV for the muscle, 73 mV for the nerve. The highest observed potential in muscle agrees with that calculated from K^+ ratios but most are considerably lower, as in nerve. Since, further, the potentials measured in seemingly healthy fibers range over tens of millivolts, which would demand great changes in K^+ ratio from fiber to fiber to account for the differences, it seems improbable that the membrane potential is entirely, or perhaps even primarily, due to the K^+ diffusion potential.

That the usual demarcation potential is but a poor measure of the membrane potential has been indicated earlier. The fall of membrane potential from normal to zero as measurements are made closer to the cut must be due to current loops through the short-circuit at the cut and, perhaps, to any decreased potential generated by the membrane in the neighborhood of the injury. That the second factor does enter, is indicated by analysis of the plot of membrane potential against distance from a cut. On simple shorting, potential would rise exponentially with distance from the cut, as for a cut cable, and a semi log plot would give a straight line. As figure 5 shows, such a straight line is obtained for distances greater than 1 to 1.5 mm from the injury; but the potential deviates sharply there and falls too rapidly at lesser dis-

tances. This indicates that, nearer than 1.5 mm, the membrane is not merely shorted by a longitudinal resistance leak but that its own battery is less active or is more shorted by a decreased transverse membrane resistance as well. This latter might be a result of the increased current flowing out through the membrane which lowers ohmic resistance (cf. Curtis and Cole, '40; Pumphrey, Schmitt and Young, '40), or of some less defined spreading injury.



These findings can be exhibited more usefully in terms of λ , the characteristic length of a fiber (Rushton), at which the transverse membrane resistance becomes equal to the longitudinal resistance sum for inside and outside ($\lambda = K \sqrt{\frac{r_t}{r_i}}$), where r_t and r_i are respectively the specific transverse resistance and the sum of the internal and external longitudinal ones. From cable theory, $V = V_o (1 - e^{-x/\lambda})$ where V_o is the original potential and V the actual potential at a distance x from the cut in the "cable." Solving for λ we have: $\lambda = \frac{-x}{\log(V_o - V) - \log V_o}$, so that plotting x against $\log(V_o - V)$ gives λ as the slope of the straight line. This is done in figure 5. The value of λ (2.8 mm) so obtained from the straight-line portion of the curve is valid; but since the equation is based on the assumption of constant transverse and longitudinal resistances, and since the curvature of the line shows that these are no longer constant in the vicinity of the cut, values obtained from the deviant portion of the

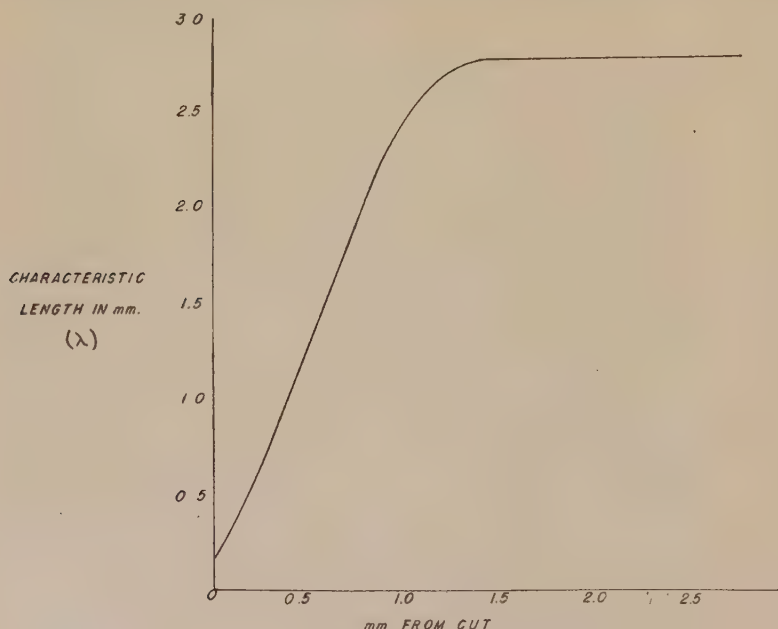


Fig. 6 Change of characteristic length with distance from cut.

curve can be considered only as indications of the way λ is decreasing and as giving "equivalent λ " values, not absolute ones. These are shown in figure 6.

The transmembrane stimulation experiments require no further discussion. A word may be added, however, on the relation of threshold to membrane potential. Several views have been advanced as to which attribute of the membrane must be changed to some critical extent for effective excitation to occur (see Katz, '39). It should be possible with this preparation to determine the relation of membrane potential to excitation threshold, as these vary with drug application, temperature, etc. Results so far have not been decisive, mainly because threshold measurements were poorly reproducible on fibers which contract with a needle in place and which are thus injured further with each twitch.

SUMMARY

1. By means of a DC amplifier, based on the new Victoreen tube, and of micropipette electrodes, membrane potentials of individual fibers of the frog sartorius have been measured. The (uncorrected) average is 62 mV; the highest value, 80 mV.

2. With the microelectrode in place, the potential usually falls, exponentially with time, on the average to about three-fourths the original value in 20 minutes. In some cases, the initial potential is maintained indefinitely.

3. Evidence is presented that these measurements are a close approximation of true membrane potential values, in contrast to the usual injury potential measurements in which an important section of the "discharge circuit" is omitted.

4. Agents known to damage the membrane are shown to cause a fall in the membrane potential. The relation of membrane potential to distance from a cut has been explored in detail.

5. Transmembrane stimulation, using the same microelectrodes, is successful only when the current acts to depolarize the membrane; 100-fold greater currents in the opposed direction are ineffective.

6. Both a minimal current density and a minimal membrane area are required for excitation. Threshold voltages are lower than those required for normal surface excitation. Time-intensity curves are of the usual type and chronaxie values are the familiar ones.

7. Evidence is presented indicating a reversal of potential during an action.

8. The relation of membrane potential to $[K^+]$, to mechanical injury, and to irritability is considered.

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